Kidney International, Vol. 53 (1998), pp. 1461–1465

Role of genetic variants of the renin-angiotensin system in chronic renal allograft injury

Arya M. Sharma, Joachim Beige, and Armin Distler

Department of Internal Medicine, Division of Endocrinology and Nephrology, Universitätsklinikum Benjamin Franklin, Free University of Berlin, Berlin, Germany

Role of genetic variants of the renin-angiotensin system in chronic renal allograft injury. In the vast majority of patients undergoing kidney transplantation, long-term success is markedly limited by a gradual decrease in graft function over time, often termed as "chronic rejection" or "chronic allograft injury." Although there have been no formal studies examining the role of genetic factors other than those related to histocompatibility for the development or progression of chronic allograft rejection, it is likely that genetic factors affecting blood pressure regulation, mesangial or vascular proliferation, or aspects of inflammatory response including thrombosis, chemotaxis, or fibrosis may play an important role in this complex syndrome. There is currently little hope that the responsible genes can be identified through sib-pair or linkage studies in families. Therefore, the study of candidate genes selected on the basis of our current understanding of the pathophysiological mechanisms involved in the chronic rejection response appears the only feasible approach. Thus far, studies have focused mainly on the role of functional genetic variants of the renin-angiotensin system on renal allograft funding. These studies, however, have not identified these variants as important determinants of renal allograft survival. Clearly, future studies will have to address the role of other variants of this system as well as genes encoding for other systems deemed to be of pathophysiological significance for the development and progression of chronic transplant injury.

During the last two decades, remarkable improvements in immunosuppression have resulted in dramatic improvements in short-term renal allograft survival. However, in the vast majority of patients undergoing kidney transplantation, long-term success is markedly limited by a gradual decrease in graft function over time. The mechanisms leading to the progressive loss of graft function, often termed "chronic rejection" or "chronic allograft injury," remain largely unknown and no effective therapy is available [1].

Thus far, both immunologic and non-immunologic factors have been implicated in the development and progression of chronic allograft injury. While the degree of HLA mismatch has not been unequivocally found to be an important determinant of chronic rejection [2, 3], the most consistent immunologic factor related to chronic allograft injury has been the occurrence of acute rejection episodes [3, 4]. Nevertheless, although the introduction of more potent immunosuppressive drugs including cyclosporine has resulted in a marked reduction in acute allograft rejection, to date this has not had a discernible effect on long-term allograft survival [1, 3].

Non-immunological factors implicated in chronic allograft injury include ischemia time, extremes of age of donor, smoking history, and the presence of hypertension, hyperlipidemia or proteinuria [1]. Despite these associations found in epidemiological cohort and cross-sectional studies, the protective value of intervention with specific anti-hypertensive, lipid-lowering, or anti-proteinuric drugs in preventing chronic allograft rejection has yet to be demonstrated.

Although there have been no formal studies examining the role of genetic factors other than those related to histocompatibility for the development or progression of chronic allograft rejection, it is likely that genetic factors play an important role in this complex syndrome [1]. Biopsy findings characteristically show vascular sclerosis with mural thickening and occlusion of renal arteries and ischemic glomeruli, interstitial infiltration by macrophages and lymphocytes, and interstitial fibrosis [5]. It is thus conceivable that genetic factors affecting blood pressure regulation, mesangial or vascular proliferation, or aspects of inflammatory response including thrombosis, chemotaxis, or fibrosis may determine the extent and progression of chronic allograft injury. These genetic factors can be active both in the host or within the transplanted kidney.

Given the complexity of the factors apparently involved in chronic rejection, there is no reason to believe that a single gene or genetic variant will act as a strong determinant of the loss of renal function by itself. It is more likely that a variety of genetic factors will play a role in determining the response to environmental factors, development of risk factors like hypertension, dyslipidemia or proteinuria, development of allograft injury, and the rate of progression (Fig. 1). As a result, a host of candidate genes could be envisioned to play a role at one or more of these steps in the development of chronic rejection. Luckily, development of end-stage renal failure remains a relatively rare event, and therefore studies in families with several members undergoing kidney transplantation are largely limited to anecdotal reports in families with hereditary renal disease. While in some instances genotyping non-affected parents may play a role in demostrating preferential transmission of deleterious alleles to affected offspring, there is currently little hope that genes determining the processes involved in chronic allograft injury can be identified through affected sibling-pair or linkage studies in families. Therefore, the study of candidate genes selected on the basis of our

Key words:: renin-angiotensin system, genetics, renal failure, transplantation, hypertension.

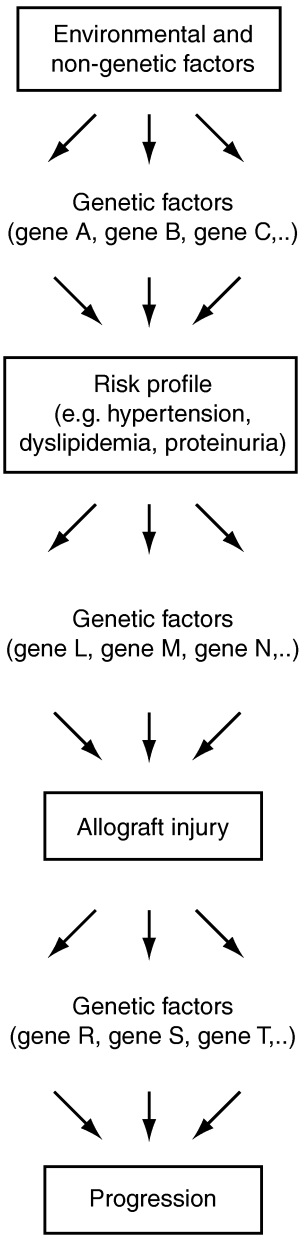

Fig. 1. Relationship between genetic factors and determinants of chronic renal allograft injury.

current understanding of the pathophysiological mechanisms involved in the chronic rejection response appears to be the only feasible approach. It is obvious that the study of functional variants of genes implicated in the development of hypertension or inflammatory response should serve as prime candidates for a role in chronic rejection.

ROLE OF THE RENIN-ANGIOTENSIN SYSTEM

Based on this line of reasoning, the components of the renin-angiotensin system are clearly candidate genes of considerable interest. Several lines of evidence do indeed suggest a role for this system in the development and progression of chronic allograft injury. Thus, hypertrophy of the juxtaglomerular apparatus is a common histological feature of chronic rejection and presumably indicates increased renin secretion [6]. Higher plasma renin activity has been reported in patients with worsening of graft function [7], and has also been implicated as a cause of post-transplant hypertension [8], an important risk factor for allograft injury [1]. The importance of the renin-angiotensin system for immune-mediated injury in renal [9], cardiac [10], and aortic [11] allografts in rats has been previously demonstrated by attenuation of injury by administration of angiotensin converting enzyme (ACE) inhibitors. Also, short-term studies in humans have demonstrated reduced proteinuria with ACE inhibition in hypertensives with renal transplants [12].

Recent studies have identified a variety of genetic variants of the components of the renin-angiotensin system [13, 14]. This article focuses on the findings derived from studies of these variants in patients with renal allograft transplantation.

ANGIOTENSINOGEN GENE

Angiotensinogen (AGT) or renin-substrate, a protein synthesized primarily in the liver but also in other tissues including the kidney, is catalyzed by renin to angiotensin I, which is subsequently converted to angiotensin II by the angiotensin-converting enzyme. Several family and sibling-pair studies have demonstrated a linkage between the angiotensinogen-gene (*AGT*) locus and essential hypertension [13, 15, 16]. Furthermore, a biallelic variant encoding either for a methionine (M) or threonine (T) at codon 235 of the mature angiotensinogen protein, associated with increased circulating angiotensinogen levels [13], has been linked to an increased risk for the development of hypertension both in Caucasian [13, 17, 18] and Japanese [19–21] humans. We have also reported that the *AGT* 235M/T-variant is associated with an early onset of hypertension in German Caucasian populations both in Berlin and Heidelberg [22]. In a recent meta-analysis we found a 20 to 40% increase in the risk for hypertension associated with the *AGT* 235T variant in Caucasians [23]. This variant of the angiotensinogen gene is now known to be in almost complete linkage disequilibrium with a biallelic variant involving the substitution of an adenine (A) for a guanine (G) nucleotide at position −6 upstream from the initiation site of transcription [24]. This −6A variant apparently binds transcription factors affecting the basal rate of transcription, thereby possibly accounting for the association between the *AGT* 235T variant and increased plasma levels of angiotensinogen.

Given that hypertension is an important risk factor for the progression of chronic allograft injury, and as many as 70% of all renal transplant recipients develop hypertension [1], it is conceivable that a variant of the angiotensinogen gene associated with essential hypertension may also increase the risk for the development of post-transplant hypertension. In rats exogenous angiotensinogen increases blood pressure under a low salt diet, demonstrating that with high renin levels, availability of angiotensinogen determines angiotensin I generation [25]. As higher levels of renin activity are found in a large proportion of renal-allograft recipients [7, 26, 27], one may expect that individuals harboring the *AGT* 235T variant would be more prone to developing post-transplant hypertension.

We recently addressed this question in a study on 269 consecutive patients undergoing kidney transplantation between 1988 and 1993 at our hospital [28]. Genomic DNA for genetic analysis was prospectively collected from all recipients and donors and the

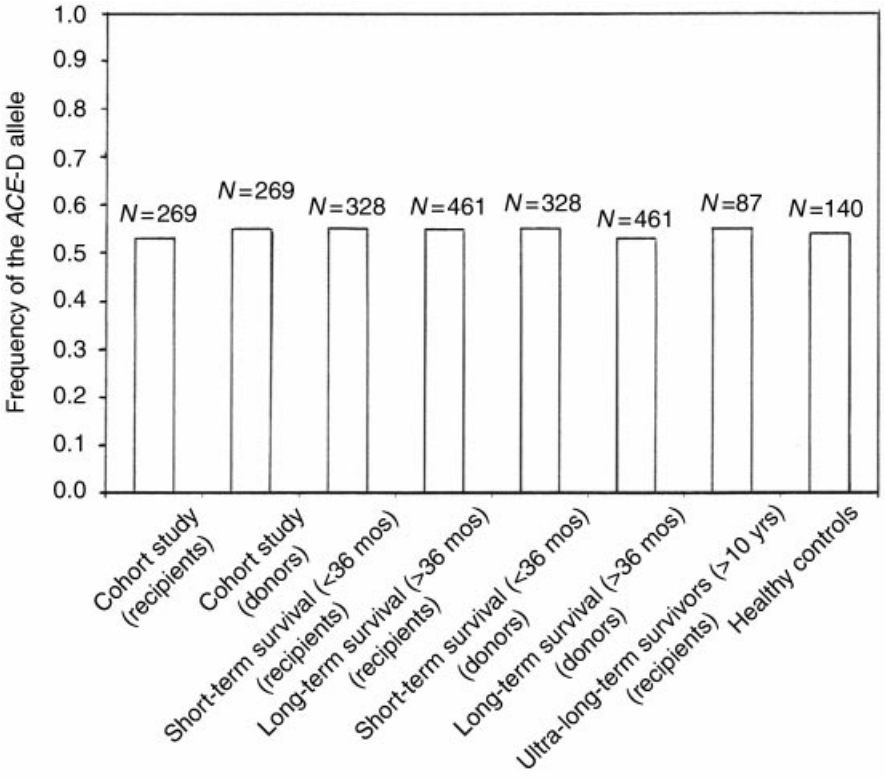

Fig. 2. Allelic frequencies of the *ACE*-D allele in donors and recipients undergoing renal tranplantation, long-term survivors of renal transplantation, and healthy controls (Adapted from [48, 49]).

presence of hypertension, and graft survival was analyzed by blinded review of all case records over a follow-up period up to 30 months. Angiotensinogen genotype was determined by a mutagenically-separated allele-specific polymerase-chain-reaction technique. While post-transplant hypertension was present in 78% of all patients, we found no relationship between either donor or recipient genotype and the presence or severity of post-transplant hypertension. Furthermore, there was no relationship between angiotensinogen genotype and graft survival during the course of the study. These findings clearly do not support the hypothesis that the M235T-variant of the angiotensinogen gene is a risk factor for the development of post-transplant hypertension. This finding may indicate that other factors including administration of cyclosporine, salt and fluid retention, or administration of glucocorticoids may have a greater impact on the development of hypertension than the angiotensinogen genotype. However, this study does not rule out that this genetic variant may predispose to the development of post-transplant hypertension or chronic rejection in a subset of patients.

ANGIOTENSIN CONVERTING ENZYME

In most tissues ACE is the key catalyst of the conversion of angiotensin I to angiotensin II. Serum and tissue levels of ACE are now known to be under genetic control [14]. Thus, individuals harboring a deletion (D) of a 289 bp *Alu* sequence located in intron 16 of the gene display higher humoral and tissue activity of this enzyme than individuals who are homozygous for the insertion (I) allele [14, 29]. Although ACE exists in a free circulating form, most of it is expressed as a transmembrane protein that is also present both in endothelial and epithelial cells in the kidney [30].

Apart from its role in the renin-angiotensin system, ACE is also the major inactivating enzyme of bradykinin, substance P, and other peptides that play important roles in several aspects of inflammatory and immunological responses [31]. Importantly, large amounts of ACE are expressed constitutively in macrophages [32], monocytes, and T-lymphocytes [33], and in the latter, *ACE* expression is also known to be under genetic control, with homozygotes for the D-allele expressing markedly higher levels of this enzyme than other individuals [33].

Recent studies have now implicated the *ACE*-D variant as a risk factor for the development of diabetic nephropathy [34–37] and an increased rate of progression of IgA nephropathy [38–40], but these findings have not been confirmed by all investigators [23, 41–45]. In 1995 Gaciong et al also reported a higher prevalence of the *ACE*-D allele (qD = 0.63) in kidney transplant recipients versus healthy age-matched blood donors (qD = 0.52), and suggested that this may be due to the fact that the *ACE*-D allele is a risk factor for the progression of renal disease [46]. In a more recent study, homozygosity for the *ACE*-D variant has also been described as a risk factor for early development of end-stage renal failure in patients with *PKD1* adult polycystic kidney disease [47]. The implication of this genetic variant in the progression of a variety of renal diseases of considerable etiological heterogeneity suggests that the role of this polymorphism may be rather unspecific, possibly affecting structural or inflammatory processes common to the majority of progressive renal disorders.

Based on these findings, we recently examined the hypothesis

that the *ACE* I/D genotype may be related either to the onset or progression of chronic allograft injury. This question was addressed in three distinct settings: a single-center cohort of 269 Caucasian patients undergoing kidney transplantation between 1988 and 1993 in whom we analyzed the appearance of biopsy-confirmed chronic rejection or allograft loss over a follow-up period of 30 months [48]; a multi-center case-control study in 328 patients with an allograft survival of less than three years (median survival 11 months) versus 461 patients with an allograft survival of at least three years (median survival 65 months) [48]; a group of 86 transplant recipients with a graft survival of at least 10 years (median survival 156 months) [49]. Neither in the cohort nor in the case control was there a significant effect of recipient or donor *ACE* genotype on transplant survival. Furthermore, the frequency of the *ACE*-D allele both in donors and recipients was similar to that reported by us and others in healthy Caucasian controls (Fig. 2). Likewise, in the patients surviving with a renal allograft for at least ten years, the frequency of the *ACE*-D allele was similar to that found in patients with early graft loss.

This lack of relationship between the *ACE*-I/D genotype is also in line with a recent report on a Dutch cohort [50] of 318 patients with at least one year of graft survival. In that study, the relative risk for graft failure over a five year follow-up period in DD recipients was 1.82 (95% CI 0.66 to 5.01) versus the II genotype. After correction for acute rejection and inclusion of patients dying with a functioning allograft, the five-year allograft failure in DD recipients was higher than in II recipients (RR 1.92; 95% CI 0.91 to 4.02), but this result was not statistically significant. These authors, therefore, likewise concluded that neither donor nor recipient *ACE*-genotype significantly affected graft survival.

Nevertheless, the lack of relationship between renal allograft survival and the *ACE* I/D genotype does not completely rule out a role for increased ACE activity in the development of chronic allograft rejection. Other variants of the *ACE* gene [51] might yet be associated with either the development of end-stage renal failure or the progression of renal injury including chronic transplant rejection, and the *ACE*-gene therefore remains an attractive candidate gene. Furthermore, given that the development of chronic transplant rejection is probably the result of a complex interaction between a variety of immunologic, genetic, and environmental factors [1], these findings do not completely rule out an importance of the *ACE* genotype in certain subsets of patients or under certain environmental conditions.

Since the *ACE*-D allele has also been associated with increased humoral and tissue activity of this enzyme [14, 29, 33], it appears worthwhile to pursue the question of whether individuals with the DD genotype profit more from therapeutic blockade of the renin-angiotensin system than other individuals. This suggestion is based on the findings of Yoshida et al [39], who demonstrated a significant reduction of proteinuria following ACE inhibition in patients with IgA nephropathy and the DD genotype, but not in patients with the ID or II genotype. Similarly, individuals harboring the D allele were found to have a larger decrease in blood pressure during ACE inhibition than II patients [52], indicating that the *ACE* genotype may significantly influence the therapeutic response to blockade of the renin-angiotensin system.

SUMMARY AND OUTLOOK

While the complexity and variability of chronic allograft rejection makes it very likely that genetic factors play a role in the development or progression of this syndrome, current studies focusing on the renin-angiotensin system have thus far not identified genetic variants of this system as important determinants. These negative findings, however, do not rule out a role for this system in the functional, inflammatory, or structural changes found in chronic rejection. Clearly, future studies will have to address the role of genetic variants of genes coding for components of other systems that may play a role in these processes. However, given our current limited understanding of the pathomechanisms involved, the host of potential candidate genes makes this a daunting, if not impossible, task. Hopefully, further experimental and clinical studies will improve our understanding of the pathophysiological mechanisms determining progressive renal allograft injury and will thus facilitate the selection of relevant candidate genes for future investigations.

Reprint requests to Prof. Dr. A.M. Sharma, Medizinische Klinik, Klinikum Benjamin Franklin, Free University of Berlin, Hindenburgdamm 30, D-12200 Berlin, Germany.
E-mail: sharma@zedat.fu-berlin.de

REFERENCES

1. Hostetter TH: Chronic transplant rejection. *Kidney Int* 46:266–279, 1994
2. Held PJ, Kahan BD, Hunsicker LG, Liska D, Wolfe RA, Port FK, Gaylin DS, Garcia JR, Agodoa LY, Krakauer H: The impact of HLA mismatches on the survival of first cadaveric kidney transplants. *N Engl J Med* 331:765–770, 1994
3. Massy ZA, Guijarro C, Wiederkehr MR, Ma JZ, Kasiske BL: Chronic renal allograft rejection: Immunologic and nonimmunologic risk factors. *Kidney Int* 49:518–524, 1996
4. Ferguson R: Acute rejection episodes–Best predictor of long-term primary cadaveric renal transplant survival. *Clin Transplant* 8:328–331, 1994
5. Solez K, Benediktsson H, Cavallo T, Croker B, Demetris AJ, Drachenberg C, Emancipator S, Furness PN, Gaber LW, Gibson IW, Gough J, Gupta R, Halloran P, Hayry P, Kashgarian M, Marcussen N, Massy ZA, Mihatsch MJ, Morozumi K, Noronha I, Olsen S, Papadimitriou J, Paul LC, Picken M, Racusen LC, et al: Report of the Third Banff Conference on Allograft Pathology (July 20–24, 1995) on classification and lesion scoring in renal allograft pathology. *Transplant Proc* 28:441–444, 1996
6. Crocker B: Pathology of the renal allograft, in *Renal Pathology*, edited by Tisher C, Brenner B, Philadelphia, Lippincott, 1989, p 1518
7. Bresticker M, Nelson J, Huang C, Wolf J, Anderson B: Plasma renin activity in renal transplant patients with hypertension. *Am J Hypertens* 4:623–626, 1991
8. Luke RG: Pathophysiology and treatment of posttransplant hypertension. *J Am Soc Nephrol* 2:S37–S44, 1991
9. Rumble J: Comparison of effects of ACE inhibition with calcium channel blockade on renal disease in model combining genetic hypertension and diabetes. *Am J Hypertens* 8:53–57, 1995
10. Paul LC, Davidoff A, Benediktsson H: Cardiac allograft atherosclerosis in the rat. The effect of histocompatibility factors, cyclosporine, and an angiotensin-converting enzyme inhibitor. *Transplantation* 57:1767–1772, 1994
11. Michel JB, Plissonnier D, Bruneval P: Effect of perindopril on the immune arterial wall remodeling in the rat model of arterial graft rejection. *Am J Med* 92:39S–46S, 1992
12. Bochicchio T, Sandoval G, Ron O, Perez Grovas H, Bordes J, Herrera Acosta J: Fosinopril prevents hyperfiltration and decreases proteinuria in post-transplant hypertensives. *Kidney Int* 38:873–879, 1990
13. Jeunemaitre X, Soubrier F, Kotelevtsev YV, Lifton RP, Williams CS, Charru A, Hunt SC, Hopkins PN, Williams RR, Lalouel JM, Corvol P: Molecular basis of human hypertension: Role of angiotensinogen. *Cell* 71:169–180, 1992

14. Tiret L, Rigat B, Visvikis S, Breda C, Corvol P, Cambien F, Soubrier F: Evidence, from combined segregation and linkage analysis, that a variant of the angiotensin I-converting enzyme (ACE) gene controls plasma ACE levels. *Am J Hum Genet* 51:197–205, 1992
15. Djurhuus MS, Henriksen JE, Klitgaard NA, Beck-Nielsen H: Obesity and coronary risk factors among South Asians. *Lancet* 337: 971, 1991
16. Caulfield M, Lavender P, Newell-Price J, Farrall M, Kamdar S, Daniel H, Lawson M, De Freitas P, Fogarty P, Clark AJL: Linkage of the angiotensinogen gene locus to human essential hypertension in African Caribbeans. *J Clin Invest* 96:687–692, 1995
17. Jeunemaitre X, Charru A, Chatellier G, Dumont C, Sassano P, Soubrier F, Ménard J, Corvol P: M235T variant of the human angiotensinogen gene in unselected hypertensive patients. *J Hypertens* 11(Suppl 5):S80–S81, 1993
18. Tiret L, Ricard S, Poirier O, Arveiler D, Cambou J-P, Luc G, Evans A, Nicaud V, Cambien F: Genetic variation at the angiotensinogen locus in relation to high blood pressure and myocardial infarction: The ECTIM study. *J Hypertens* 13:311–317, 1995
19. Hata A, Namikawa C, Sasaki M, Sato K, Nakamura T, Tamura K, Lalouel J-M: Angiotensinogen as a risk factor for essential hypertension in Japan. *J Clin Invest* 93:1285–1287, 1994
20. Iwai N, Shimoike H, Ohmichi N, Kinoshita M: Angiotensinogen gene and blood pressure in the Japanese population. *Hypertension* 25:688–693, 1995
21. Nishiuma S, Kario K, Kayaba K, Nagio N, Shimada K, Matsuo T, Matsuo M: Effect of the angiotensinogen gene $Met^{235}\rightarrow Thr$ variant on blood pressure and other cardiovascular risk factors in two Japanese populations. *J Hypertens* 13:717–722, 1995
22. Schmidt S, Sharma AM, Zilch O, Beige J, Walla Friedel M, Ganten D, Distler A, Ritz E: Association of M235T variant of the angiotensinogen gene with familial hypertension of early onset. *Nephrol Dial Transplant* 10:1145–1148, 1995
23. Kunz R, Kreutz R, Beige J, Distler A, Sharma AM: Association between the angiotensinogen 235T-variant and hypertension in Caucasians: A systematic review and methodological appraisal. *Hypertension* 30:1331–1337, 1997
24. Inoue I, Nakajima T, Williams CS, Quackenbush J, Puryear R, Powers M, Cheng T, Ludwig EH, Sharma AM, Hata A, Jeunemaitre X, Lalouel JM: A nucleotide substitution in the promoter of human angiotensinogen is associated with essential hypertension and affects basal transcription in vitro. *J Clin Invest* 99:1786–1797, 1997
25. Menard J, El Amrani AI, Savoie F, Bouhnik J: Angiotensinogen: An attractive and underrated participant in hypertension and inflammation. *Hypertension* 18:705–707, 1991
26. First MR, Neylan JF, Rocher LL, Tejani A: Hypertension after renal transplantation. *J Am Soc Nephrol* 4(Suppl):S30–S36, 1994
27. Curtis JJ: Hypertension following kidney transplantation. *Am J Kidney Dis* 23:471–475, 1994
28. Beige J, Weber S, Engeli S, Offermann G, Distler A, Sharma AM: Angiotensinogen-M235T genotype and post-transplant hypertension. *Nephrol Dial Transplant* 11:1538–1541, 1995
29. Rigat B, Hubert C, Alhenc Gelas F, Cambien F, Corvol P, Soubrier F: An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest* 86:1343–1346, 1990
30. Rosenberg ME, Smith LJ, Correa Rotter R, Hostetter TH: The paradox of the renin-angiotensin system in chronic renal disease. *Kidney Int* 45:403–410, 1994
31. Erdos EG: Angiotensin I converting enzyme and the changes in our concepts through the years. Lewis K Dahl memorial lecture. *Hypertension* 16:363–370, 1990
32. Friedland J, Setton C, Silverstein E: Induction of angiotensin converting enzyme in human monocytes in culture. *Biochem Biophys Res Commun* 83:843–849, 1978
33. Costerousse O, Allegrini J, Lopez M, Alhenc Gelas F: Angiotensin I-converting enzyme in human circulating mononuclear cells: Genetic polymorphism of expression in T-lymphocytes. *Biochem J* 290:33–40, 1993
34. Marre M, Bernadet P, Gallois Y, Savagner F, Guyene TT, Hallab M, Cambien F, Passa P, Alhenc Gelas F: Relationships between angiotensin I converting enzyme gene polymorphism, plasma levels, and diabetic retinal and renal complications. *Diabetes* 43:384–388, 1994
35. Mizuiri S, Hemmi H, Inoue A, Yoshikawa H, Tanegashima M, Fushimi T, Ishigami M, Amagasaki Y, Ohara T, Shimatake H, Hasegawa A: Angiotensin-converting enzyme polymorphism and development of diabetic nephropathy in non-insulin-dependent diabetes mellitus. *Nephron* 70:455–459, 1995
36. Doi Y, Yoshizumi H, Yoshinari M, Iino K, Yamamoto M, Ichikawa K, Iwase M, Fujishima M: Association between a polymorphism in the angiotensin-converting enzyme gene and microvascular complications in Japanese patients with NIDDM. *Diabetologia* 39:97–102, 1996
37. Ohno T, Kawazu S, Tomono S: Association analyses of the polymorphisms of angiotensin-converting enzyme and angiotensinogen genes with diabetic nephropathy in Japanese non-insulin-dependent diabetics. *Metabolism* 45:218–222, 1996
38. Yorioka T, Suehiro T, Yasuoka N, Hashimoto K, Kawada M: Polymorphism of the angiotensin converting enzyme gene and clinical aspects of IgA nephropathy. *Clin Nephrol* 44:80–85, 1995
39. Yoshida H, Mitarai T, Kawamura T, Kitajima T, Miyazaki Y, Nagasawa R, Kawaguchi Y, Kubo H, Ichikawa I, Sakai O: Role of the deletion of polymorphism of the angiotensin converting enzyme gene in the progression and therapeutic responsiveness of IgA nephropathy. *J Clin Invest* 96:2162–2169, 1995
40. Harden PN, Geddes C, Rowe PA, McIlroy JH, Boulton Jones M, Rodger RS, Junor BJ, Briggs JD, Connell JM, Jardine AG: Polymorphisms in angiotensin-converting-enzyme gene and progression of IgA nephropathy. *Lancet* 345:1540–1542, 1995
41. Schmidt S, Schöne N, Ritz E, Diabetic Nephropathy study Group: Association of ACE gene polymorphism and diabetic nephropathy. *Kidney Int* 47:1176–1181, 1995
42. Tarnow L, Cambien F, Rossing P, Nielsen FS, Hansen BV, Lecerf L, Poirier O, Danilov S, Parving HH: Lack of relationship between an insertion/deletion polymorphism in the angiotensin I-converting enzyme gene and diabetic nephropathy and proliferative retinopathy in IDDM patients. *Diabetes* 44:489–494, 1995
43. Chowdhury TA, Dronsfield MJ, Kumar S, Gough SLC, Gibson SP, Khatoon A, MacDonald F, Rowe BR, Dunger DB, Dean JD, Davies SJ, Webber J, Smith PR, MacKin P, Marshall SM, Adu D, Morris PJM, Todd JA, Barnett AH, Boulton AJM, Bain SC: Examination of two genetic polymorphisms within the renin-angiotensin system: No evidence for an association with nephropathy in IDDM. *Diabetologia* 39:1108–1114, 1996
44. Fukushima T, Nomura S, Kawai S, Osawa G: ACE genotype and progression of IgA nephropathy. *Lancet* 346:571, 1995
45. Ringel J, Beige J, Kunz R, Distler A, Sharma AM: Genetic variants of the renin-angiotensin system, diabetic nephropathy and hypertension. *Diabetologia* 40:193–199, 1997
46. Gaciong ZA, Religa P, Placha G, Rell K, Paczek L: ACE genotype and progression of IgA nephropathy. *Lancet* 346:570, 1995
47. Baboolal K, Ravine D, Daniels J, Williams N, Holmans P, Coles GA, Williams JD: Association of the angiotensin I converting enzyme gene deletion polymorphism with early onset of ESRF in *PKD1* adult polycystic kidney disease. *Kidney Int* 52:607–613, 1997
48. Beige J, Scherer S, Weber A, Engeli S, Offermann G, Opelz G, Distler A, Sharma AM: Angiotensin-converting enzyme genotype and renal allograft survival. *J Am Soc Nephrol* 8:1319–1323, 1996
49. Beige J, Offermann G, Distler A, Sharma AM: Angiotensin-converting-enzyme insertion/deletion genotype and long-term allograft survival. *Nephrol Dial Transplant* (in press)
50. Boeckoelofs J, Stegeman CA, Navie GJ, Scheffer H, Tegzess AM, De Zeeuw D, De Jong PE: Is donor or recipient ACE-genotype associated with long-term graft survival after renal transplantation? (abstract) *Kidney Int* 52:559, 1997
51. Villard E, Tiret L, Visvikis S, Rakotovao R, Cambien F, Soubrier F: Identification of new polymorphisms of the angiotensin I-converting enzyme (ACE) gene, and study of their relationship to plasma ACE levels by two-QTL segregation-linkage analysis. *Am J Hum Genet* 58:1268–1278, 1996
52. Hingorani AD, Jia HY, Stevens PA, Hopper R, Dickerson JEC, Brown MJ: Renin-angiotensin system gene polymorphisms influence blood pressure and the response to angiotensin converting enzyme inhibition. *J Hypertens* 13:1602–1609, 1995